# PROTEIN COMPONENTS AND ANTIGENS OF THE VENEZUELAN EQUINE ENCEPHALOMYELITIS VIRUS

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# Protein components and antigens of the Venezuelan equine encephalomyelitis virus

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Abstract: The protein composition of highly purified Venezuelan equine encephalomyelitis virus (VEE) was studied by the methods of electrophoresis in polyacrilamide gel and double diffusion in agar. Coincidence of the results obtained by the two indicated methods permits the conclusion to be drawn that three virus-specific proteins are present in the structure of VEE virions.

Despite the fact that group A arboviruses have in recent years become a prevalent model for studying the structure, composition, and rules governing the multiplication of RNA-containing viruses, the question of the protein composition of the viruses of this group is even today under discussion. The molecular weight of the RNA-viruses of this group comprises about $2 \times 10^6$ daltons /15/; it may be expected that this group contains information for the synthesis of at least 4 proteins /6/. It has been shown by the method of electron microscopy that the arbovirus virions consist of an external lipoproteid shell, a basal membrane, and an internal body containing RNA and protein. The external shell is linked with such biological properties of the virus as the capacity to induce hemagglutination and hemolysis of the erythrocytes /9,11/. By the method of electrophoresis in polyacrilamide gel, it has been established that the composition of the Sindbis and Western equine encephalomyelitis viruses includes at least 2 proteins /13/. At the same time, Friedman /6/ discovered at least 3 proteins in the structure of the Semliki forest virus, 2 of them

belonging to the composition of 140S-particles isolated from infected cells. The presence of 2-3 antigen proteins in the composition of arboviruses has also been shown by the method of immunodiffusion in agar gel /7,8/. The different number of structural proteins can be explained not only by the structural particularities of individual representatives of this new group of viruses, but also by the features and potentialities of the methodic procedures used by different authors.

In the present work are presented data concerning the proteins of the Venezuelan equine encephalomyelitis virus, obtained by the methods of electrophoresis in agarose-polyacrilamide gel and immunodiffusion in agar.

Materials and methods. VEE virus (strain SPF) was neutralized in initially trypsinized chick fibroblasts (spiner cluture) in medium number 199 with 0.5% warmed beef serum or in cells (mouse fibroplasts), which were cultivated in the form of a monolayer in medium number 199 with 10% beef serum.

Obtaining a labelled virus. Chlorella $C^{14}$-hydrolizate (Association "Izotop," Leningrad) was used for labelling the virus proteins; it was introduced into the accumulation medium 5 microcuries/ml at a time immediately after infection of the cells /2/.

Concentration and refinement of the virus were described earlier /1/.

Electrophoresis. Preparations of purified virus (fractions from a gradient of cesium chloride with a density of 1.25-1.23 g/cm$^3$) were solubilized (mixture: 1/10 volume of the material glacial acetic acid, 1/25% sodium dodecyl sulfate - SDS, 0.5-1 M urea, 0.8-1% mercaptoethanol, and 0.0125 M ethylenediaminetetra - acetate (EDTA) - final concentrations indicated), were dialyzed and were subjected to electrophoresis in 5% agarose-polyacrylic gel according to the method of Summers et al. /14/ in the Polyanalyst (USA) apparatus. 100-150 micrograms of the investigated material were introduced into each tube.

Immune sera. Rabbits were subjected to sixfold immunization (with a 1-week interval) by VEE virus grown in a culture of chick fibroblasts or in L cells. Anticell sera to the material of the host were obtained by the same method.

The titer of the antivirus sera was equal to 16,000 - 20,000 in the hemagglutination-inhibition test, the titer of the anticell sera was 640-1280 in the complement fixation test.

Antigens. A purified virus, expressed in terms of chick and mouse cells (VEE-ch.f. and VEE-L, respectively). The virus was destroyed by sodium desoxylate /4/ and SDS /10/; the latter was removed by the addition of a saturated solution of KCl, centrifuging and subsequent dialysis.

Used as the cell antigen was a 10% extract of normal chorionallantoic membrane of a chick embryo (NCAO), prepared on the basis of a transbuffer /?/ physiological solution, for a chick fibroblast homogenate obtained in the same manner.

Reaction of double (radial) diffusion in agar was conducted within the first 24 hours at a temperature of 37° with subsequent continuation at room temperature at 1.2% agar gel (Difco agar), prepared on the basis of a 0.07-M phosphate buffer (sodium salt), pH 7.0.

Results. Electrophoresis in gel. The method of electrophoresis in polyacrilamide gel makes it possible to determine the number of individual polypeptide chains in the form of monomers in a mixture consisting of several different proteins; this also includes viruses. Preliminary treatment of viruses by a mixture of the indicated composition brings about dissociation of the virus proteins. With electrophoresis of material treated in this manner, an electrogram will always disclose 3 peaks, containing about 80-85% of the entire radioactivity of the gel ( See Fig. 1. )

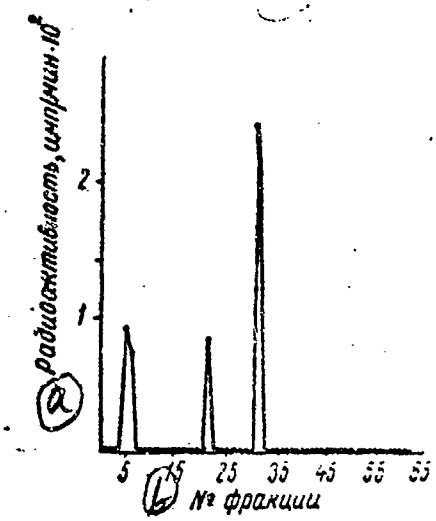


Figure 1. Proteins of VEE virus in agarose-polyacrilamide gel.

Key to Figure 1. a) radioactivity, pulses/min·$10^2$; b) number of fractions.

Identification of the peaks and the value of the molecular weights for each of them are described in reference /2/.

Method of double diffusion in agar. The quantity of antigen proteins in the structure of the VEE virus was determined by the method of double diffusion (radial) in agar gel. Highly purified preparations of the virus were used, grown in cells of different origin, the virus concentration in both cases being the same ($5 \cdot 10^8$ CBU/ml). Overall data of the experiment series are presented in the table and in Figures 2 and 3.

Table. Combined data on study of the antigen structure of VEE by the method of double diffusion (radial) in agar gel

| Сыворотка | Число полос преципитации с антигенами | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | VEE-ч. ф. | | | | VEE-L | | | | |
| | целый (1) | разрушенный | | | целый (5) | разрушенный | | | экстракт НХАО (9) |
| | | ДСН (2) | ДСН (3) | ДСН (4) | | ДСН (6) | ДСН (7) | ДСН (8) | |
| Против VEE-ч. ф. (A) | 2 | 2 | 3 | 3 | 2 | 2 | 3 | 2 (3) | 0 (1) |
| » VEE-ч. ф. истощенного НХАО (B1) | 2 | 2 | 2 | 2 | 1 (2) | 2 | 3 | 2 | 0 |
| » VEE-L (B) | 1 | 1 | 3 | 3 | 1 | 1 | 3 | 2 (3) | 0 |
| » НХАО (C) | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 (2) |

Key to table: a) serum; b) VEE-Ch.f. exhausted by NCAO; c) number of precipitation bands with antigens; d) integral; e) destroyed by; f) extract of NCAO (9)

Note. $SDS^{+}$ - treated by KCl.



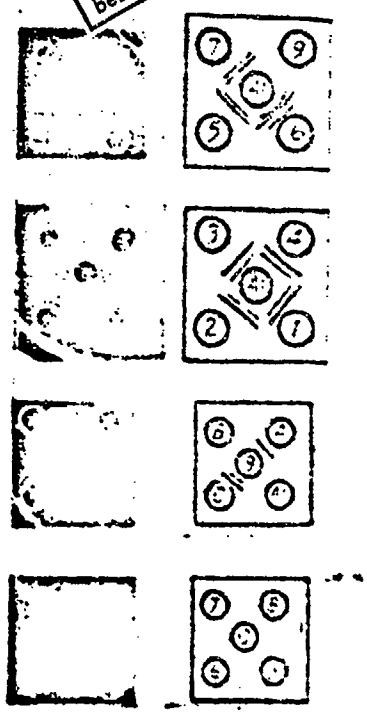

Figure 2. Development of precipitating antigens in the structure of VEE-Ch.f. and VEE-L by the method of double diffusion (radial) in agar gel.

A and Al - sera against VEE-Ch.f. with and, respectively, without exhaustion by NCAO; C - against NCAO extract. 1 - integral antigen; 2 - destroyed by DSO; 3 and 4 - SDS with and, respectively, without treatment of VEE-Ch.f. by KCl; 5 - integral; 6 - destroyed by DSO; 7 and 8 - SDS with and, respectively, without treatment of VEE-L by KCl; 9 - extract of NCAO.

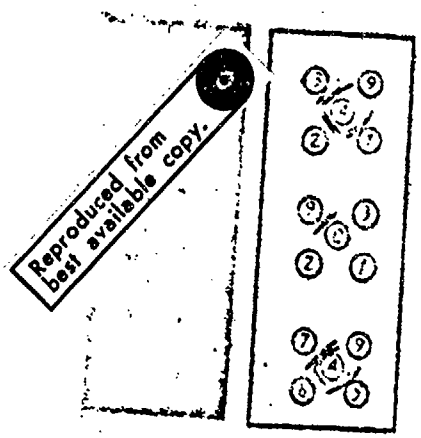


Figure 3. Identification of virus-specific antigens of VEE-Ch.f. and VEE-L virions. Designations are the same as in figure 2.

With integral preparations of VEE-Ch.f. and VEE-L, and with those destroyed by DSO, the serum against VEE-Ch.f. yielded 2 precipitating lines, one of which was formed already after 4-5 hours, and the other by 8-10 hours of incubation near the point of introduction of the antigen. Destruction of the virus by SDS brought about the determination of an additional third band, which developed 18-24 hours after the start of the experiment, at the point of introduction of the immune serum. Exhaustion of this serum by host-cell material was in some cases accompanied also by the disappearance of this precipitation line. The constant appearance of three bands when using a destroyed VEE-L virus as an antigen testifies rather to its virus-specific nature, with account taken of precise control over the absence of antibodies against components of the host in the absorbed immune serum.

In the control the anticell serum, forming a single clear precipitation line with various dilutions of a specific antigen, did not bring about the formation of precipitating lines with the virus antigen of the tube types.

Analogous results were obtained with the use of anti-VEE-L serum.

Discussion. The use of two methods of protein electrophoresis, in polyacrilamide gel and double diffusion in agar, permits the conclusion to be drawn that three proteins possessing antigenic properties are present in the composition of highly purified virions. Similar results were obtained by Horzinek with the same virus, which was studied by the method of immunoelectrophoresis. It has already been noted that three proteins were discovered by Friedman /6/ as well, in the composition of the Semliki forest virus.

The detection of only two proteins in the composition of a number of other representatives of group A arboviruses may, in our view, be due to differences in the procedural approaches employed by the researchers for analysis of the arbovirus composition, and the methods and degree of purification, as well as to structural features of the various representatives included in group A. Apparently, the same considerations should be taken into account when evaluating the data of Rott et al. /12/ concerning the presence of cellular-origin antigens in the composition of the Sindbis virus.

The method of inducing a state of immunological tolerance to host-cell antigens is a highly sensitive test for the determination of species-specific cellular antigens in the structure of viruses. Such experiments with the VEE virus /3/, as well as the data presented in the present work, permit the conclusion to be drawn that proteins discovered in the composition of the virus are of a virus-specific nature.

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## PROTEIN COMPONENTS AND ANTIGENS OF VENEZUELAN EQUINE ENCEPHALOMYELITIS VIRUS

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The protein composition and antigenic properties of purified Venezuelan equine encephalomyelitis virus (VEE) were studied by means of electrophoresis in polyacrylamide gel and double diffusion in agar gel. In the latter experiments, purified virus grown in chick embryo fibroblast cultures and L cells was used and rabbit antisera for them. The results obtained permit a conclusion on the presence in the structure of VEE virions of three proteins possessing antigenic properties and having virus-specific origin.